Carcinogenesis vol.8 no.11 pp.1741–1747, 1987

Distribution and metabolism of *N'*-nitrosonornicotine in the miniature pig

Lennart Domellöf[1,2], Magnus Andersson[1,2], Hans Tjälve[3], Susan Veals[4], Neil Trushin[4] and Stephen S.Hecht[4]

[1]Department of Surgery, University Hospital, Umeå, [3]Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, The Swedish University of Agricultural Sciences, Uppsala, Sweden, and [4]Division of Chemical Carcinogenesis, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA

[2]Present address: Department of Surgery, Örebro Medical Center Hospital, Regionsjukhuset, S-70185, Örebro, Sweden

The distribution and metabolism of [5-^{3}H]*N'*-nitrosonornicotine ([5-^{3}H]NNN) was studied in three 18-day-old miniature pigs. [5-^{3}H]NNN was administered by intracardiac administration into the right ventricle of the heart to mimic uptake by the lung. Whole body autoradiograms taken 15–220 min after treatment showed high levels of radioactivity in the mandibular and parotid salivary glands, Harder's gland, lacrimal glands, glands of the snout and respiratory part of the nasal cavity, and the melanin of the eyes and skin. Bound radioactivity was most abundant in the nasal mucosa and liver. Analysis of tissues by h.p.l.c. showed the presence of high levels of [5-^{3}H]NNN in the mandibular glands and Harder's gland. Levels of [5-^{3}H]NNN and its metabolites were determined in arterial and venous serum, 0.5–220 min after injection . The disappearance of [5-^{3}H]NNN from serum was biphasic. 4-Oxo-4-(3-pyridyl) butyric acid, a metabolite of [5-^{3}H]NNN resulting from 2'-hydroxylation, which is a suspected activation pathway, was detected 0.5 min after injection and appeared to reach a steady state 2–220 min after injection. 4-Hydroxy-4-(3-pyridyl)butyric acid, from 5'-hydroxylation of [5-^{3}H]-NNN, and norcotinine, from denitrosation, were also rapidly formed. These experiments are the first in which the appearance of NNN metabolites in blood has been measured. The ratio of 2'-hydroxylation to 5'-hydroxylation varied from 0.27 to 0.60 in arterial serum and from 0.33 to 0.49 in venous serum in the period from 2–220 min. [5-^{3}H]NNN accumulated in the stomach contents such that its levels were greater than those in arterial or venous serum, 60 min after injection. The results of this study demonstrate that the miniature pig is a useful model for the investigation of nitrosamine metabolism and indicate some similarities and differences in metabolism and distribution compared with the rat.

Introduction

N'-Nitrosonornicotine (NNN*), which is present in relatively high concentrations in unburned tobacco and tobacco smoke, is considered to be one of the most important carcinogens associated with tobacco-related cancer in man. It causes tumors of the esophagus and nasal cavity in rats, trachea and nasal cavity in Syrian golden hamsters, and lung in A/J mice (1). Extensive studies of the distribution and metabolism of NNN have been carried out in rodents (1–3). These studies have elucidated the major metabolic pathways of NNN and have demonstrated the accumulation and persistence of NNN and certain metabolites in various tissues. The rodent models are convenient and allow comparison of the results of metabolic studies to those of carcinogenicity assays. However, alternative models are useful in extending experiments on carcinogen metabolism and disposition in order to allow more meaningful extrapolations to humans. The pig has several advantages including the similarity of its gastrointestinal tract to that of humans, and its large size which allows sampling of fluids and tissues in a manner which is not always feasible in rodents (4,5). In the present study, we have examined the disposition and metabolism of NNN in the miniature pig, in order to provide a framework for further investigations of tobacco-specific nitrosamines in this animal model.

*Abbreviation: NNN, *N'*-nitrosonornicotine.

Materials and methods

Chemicals

[5-^{3}H]NNN was synthesized by tritium exchange of 5-bromonornicotine followed by nitrosation and purification by preparative thin-layer chromatography (6). Its purity was >99% according to analysis by h.p.l.c. It was diluted to a specific activity of 200 mCi/mmol with unlabeled NNN.

Animal treatments

Three 18-day-old minipigs from Astra (Södertälje, Sweden) were used for the experiment: pig 1, female, 2.6 kg; pig 2, male, 3.1 kg; and pig 3, female, 2.8 kg. After careful induction of pentobarbital anaesthesia, a tracheostomy was performed and the animals were attached to a volume cycled ventilator. Ventilation was performed by oxygen-enriched air, with fraction of inspired oxygen = 0.3. Anaesthesia was maintained by continuous i.v. pentobarbital (20 mg/kg/h). End-tidal CO_2 was maintained at ~4.0 kPa. Under fluoroscopic control, a catheter was placed in the right ventricle of the heart for intrapulmonary administration of [5-^{3}H]NNN. Catheters were placed in the thoracic aorta and in the jugular vein for blood sampling. A gastric tube was used for sampling of gastric juice.

Pigs 1 and 2 were given 125 μCi/kg (0.63 μmol/kg; 110 μg/kg [5-^{3}H]NNN and pig 3 250 μCi/kg (1.25 μmol/kg; 221 μg/kg) [5-^{3}H]NNN by intracardiac administration. Arterial and venous blood samples were collected at intervals after administration as noted in the text. The minipigs were sacrificed by a toxic dose pentobarbital at intervals after injection of [5-^{3}H]NNN as follows: pig 1, 15 min; pig 2, 60 min; pig 3, 220 min. Urine, bile and tissues were collected at sacrifice.

Whole-body autoradiography

After sacrifice, the pigs were embedded in carboxymethyl cellulose and mounted on microtome stages, immersed and rapidly frozen in hexane cooled with dry-ice to −78°C. Thin sections (20 μm) through the whole animals attached to tape (no. 810 or 688, 3M Co.), were cut (7). The sectioning of the animals for the whole-body autoradiography was performed on the left half of the bodies, and the other half was used for tissue analysis. After freeze-drying at −20°C, the sections were slowly brought to room temperature. Twenty freeze-dried sections (on tape no. 810) from each animal were apposed to X-ray film (Industrex C, Kodak) without further treatment. Twenty additional sections (on tape no. 688) from each animal were extracted with 5% trichloroacetic acid, distilled H_2O, methanol and heptane (2 min extraction in each solvent) before they were apposed to film. The washing procedure removes material which is not firmly bound to the tissues. After exposure periods of 10–15 months, sections and films, were separated and the films were developed under standardized conditions, fixed, rinsed and dried.

Serum analysis

Serum (0.1 ml) was mixed with methanol (0.3 ml) in order to precipitate protein. The precipitate was separated by centrifugation at 6000 r.p.m. The supernatant was separated and the precipitate was washed with 0.2 ml of methanol. After centrifugation the methanol supernatants were combined and centrifuged

PM3001192370

Source: https://www.industrydocuments.ucsf.edu/docs/krwk0001

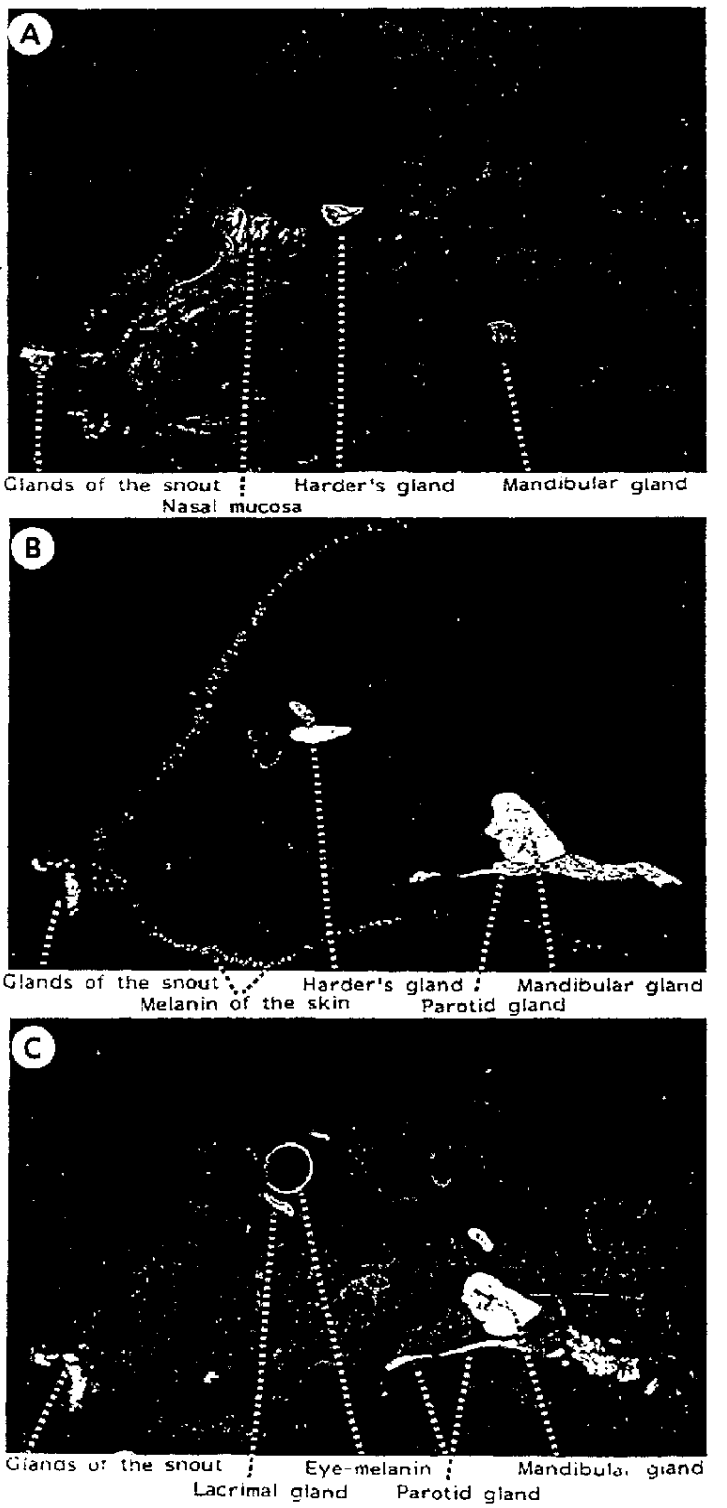

Fig. 1. Details of whole-body autoradiograms of freeze-dried non-extracted tissue-sections of miniature pigs killed 15 min (A), 60 min (B) and 220 min (C) after intracardial injections of [5-^{3}H]NNN.

PM3001192371

Source: https://www.industrydocuments.ucsf.edu/docs/krwk0001

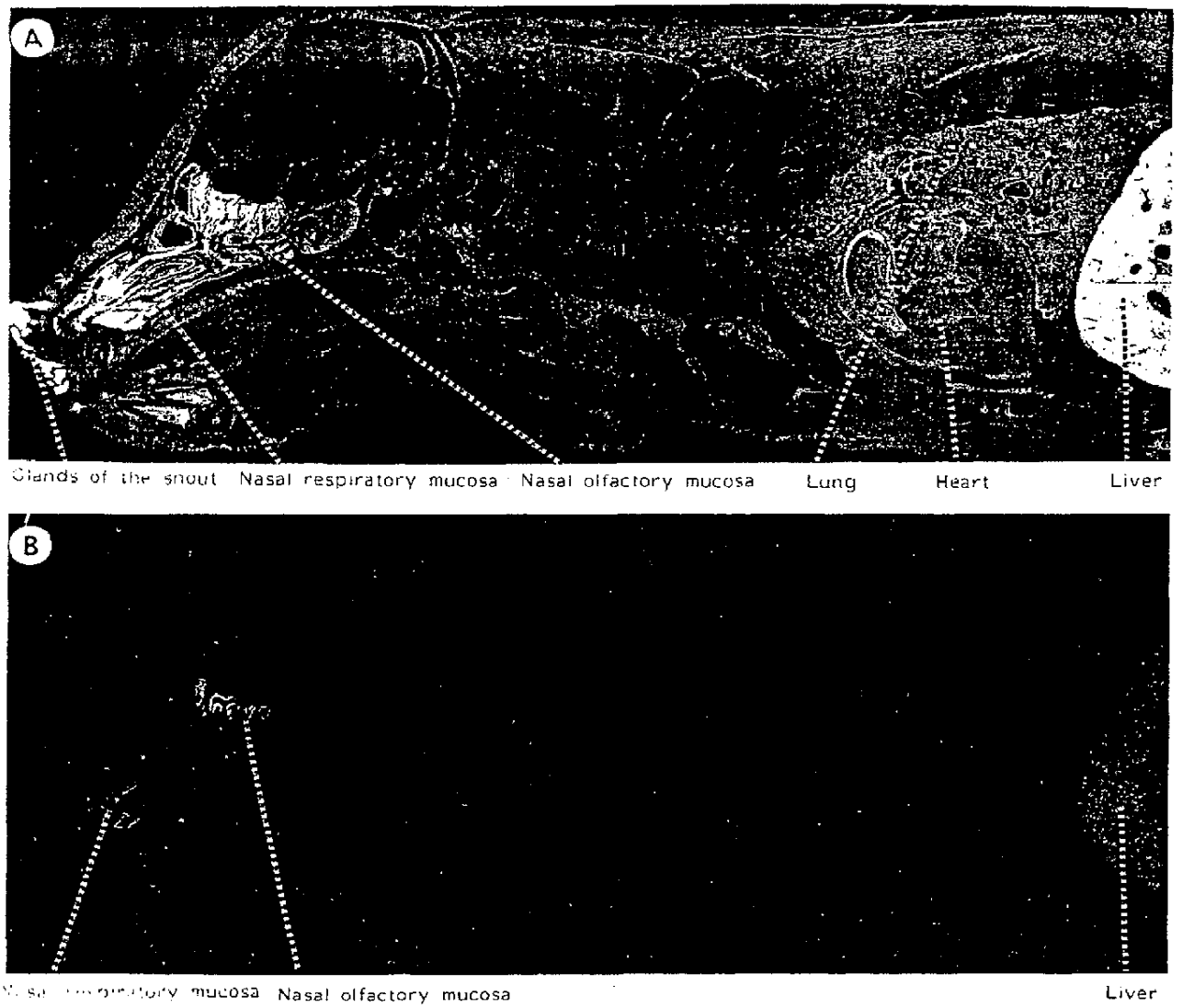

Fig. 2. Whole-body autoradiograms of a miniature pig killed 220 min after an intracardial injection of [5-^{3}H]NNN. **Panel A** is an autoradiogram of a freeze-dried non-extracted tissue-section. **Panel B** is an autoradiogram of a tissue-section adjacent to A, which was extracted with trichloroacetic acid, H_2O and organic solvents before the autoradiographic exposure.

again for 10 min to remove any traces of protein. The supernatant was filtered through a 0.45 μm h.p.l.c. grade filter and an aliquot was analyzed by scintillation counting. The remainder was concentrated to less than 0.02 ml with a stream of N_2. The residue was redissolved in 0.1 ml of H_2O. The solution was tested for protein prior to analysis by h.p.l.c. for NNN and its metabolites.

Tissue analysis

Tissue samples were homogenized in 1 ml of cold 0.1 M HCl. The homogenate was transferred to a 10-ml centrifuge tube and washed with 2 ml of 0.1 M HCl. The mixture was centrifuged at 10 000 r.p.m. for 10 min. The supernatant was removed and the pellet was washed with another 2 ml of 0.1 M HCl. The combined HCl solutions were centrifuged again and the precipitate separated. The combined precipitates were lyophilized and weighed. The pH of the supernatant was adjusted to 7.0 with 1 M NaOH. After addition of 3:1 methanol:H_2O, the mixture was centrifuged at 4000 r.p.m. for 10 min. The supernatants were combined, filtered, and concentrated to dryness under reduced pressure. The residue was redissolved in 2 ml of H_2O and a 0.1 ml aliquot was analyzed by h.p.l.c.

Urine and bile analysis

These were prepared for analysis as described (8) except that the final sample was brought to 5.0 ml with H_2O prior to h.p.l.c. analysis.

Stomach contents analysis

Aliquots of 0.4 ml were neutralized with 1 M NaOH and twice the volume of methanol was added. The resulting mixture was centrifuged at 5000 r.p.m. for 15 min. The supernatant was removed and the precipitate was washed with 1 ml methanol. The latter was centrifuged and the supernatants were combined, centrifuged and filtered. Methanol was added repeatedly until no further precipitation occurred. The filtrates were concentrated to dryness and 0.8 ml phosphate buffer, pH 7.0 was added. After sonication for 2 min the solution was filtered and analyzed by h.p.l.c.

H.p.l.c. analysis

Samples were mixed with standard NNN metabolites and analyzed on a 4.6 mm by 12.5 cm HS-5-C18 5-μm octadecylsilane bonded phase column (Perkin-Elmer, Norwalk, CT) using a pH 6.0, methanol–H_2O gradient as previously described (9). Metabolites were identified by coelution of radioactivity with standards, detected by their absorption at 254 nm. The identities of the metabolites were confirmed by analyzing some samples on two 23.9 mm by 30 cm Bondapak-C18 columns (Millipore, Waters Division, Milford, MA) with a pH 4.0, methanol–H_2O gradient as described (8).

Metabolites were quantified by the radioactivity in the appropriate peaks, cor-

PM3001192372

Source: https://www.industrydocuments.ucsf.edu/docs/krwk0001

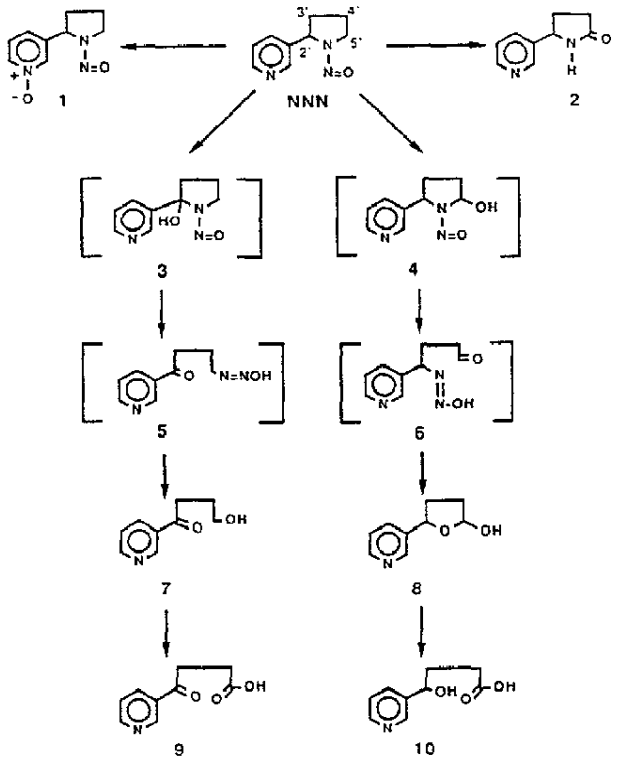

Fig. 3. Major pathways of NNN metabolism.

rected for losses during processing of the samples. This was determined by dividing the total d.p.m. which eluted from h.p.l.c. by the total d.p.m. in the original sample, as determined by scintillation counting. Recoveries ranged from 60 to 90%.

Results

Whole-body autoradiograms obtained from freeze-dried sections of pig 1 showed homogeneous labeling of most tissues of the body. The radioactivity in the blood was at a level about similar to the homogenously distributed labeling. Radioactivity markedly exceeding this level was observed in the mandibular and parotid salivary glands, Harder's gland, lacrimal glands, glands of the snout and of the respiratory part of the nasal cavity, and the melanin of the eyes and the skin (Figure 1A). Also exceeding the homogenously distributed labeling, although lower than in the tissues mentioned above, were the levels of radioactivity in the liver, the nasal olfactory and respiratory mucosa, the adrenal cortex and the kidneys. There was in addition high radioactivity in the contents of the stomach and in the urine. In the sections washed with trichloroacetic acid, H_2O and organic solvents before the autoradiography, weak radioactivity was retained in the liver, the nasal olfactory and respiratory mucosa and the pigmented tissues. The labeling of other tissues was removed by the washing procedure.

The whole-body autoradiograms of the freeze-dried sections of pig 2 (Figure 1B) showed the same general characteristics as described for pig 1. The levels of bound radioactivity in the liver and the nasal mucosa in the washed sections of pig 2 were somewhat higher than in pig 1.

In pig 3, which was killed 220 min after injection of [5-^{3}H]-NNN, autoradiography of the freeze-dried sections showed a general decrease in the labeling of most tissues, compared with

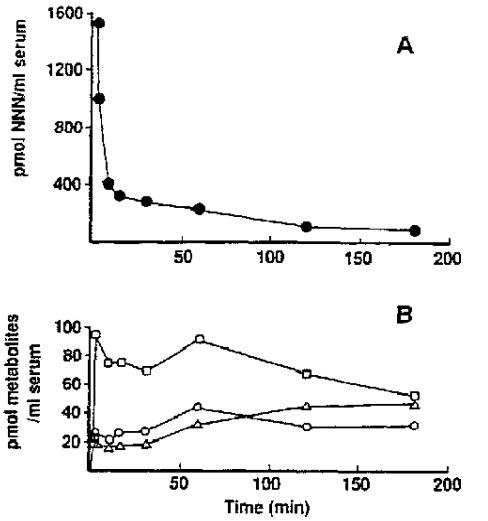

Fig. 4. Levels of NNN (A) and its metabolites (B) hydroxy acid *10* (□), keto acid *9* (○), and norcotinine *2* (△) in arterial serum at intervals after treatment of a miniature pig by intracardial injection of [5-^{3}H]NNN.

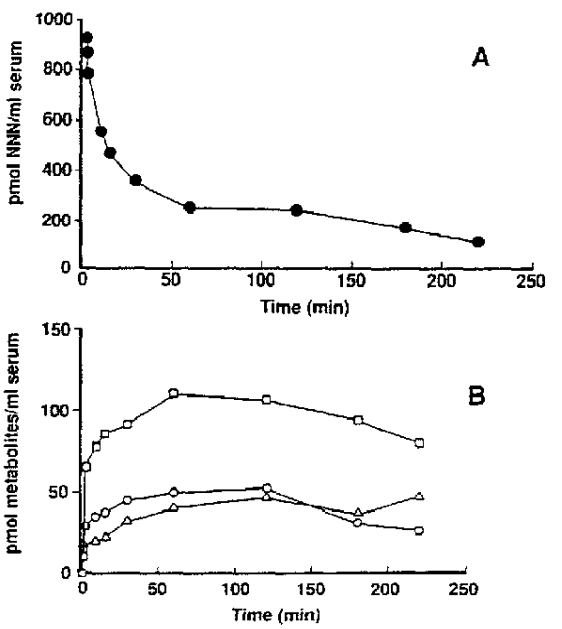

Fig. 5. Levels of NNN (A) and its metabolites (B) hydroxy acid *10* (□), keto acid *9* (○), and norcotinine *2* (△) in venous serum at intervals after treatment of a miniature pig by intracardial injection of [5-^{3}H]NNN.

pigs 1 and 2 which were killed at 15 and 60 min. However, the characteristics of the distribution patterns were similar to those observed in pigs 1 and 2 (Figure 1C). In washed sections of pig 3, the retention of radioactivity in the liver and the nasal mucosa was more marked than at the shorter survival intervals. Comparisons of unwashed and washed sections are shown in Figure 2.

Major known pathways of NNN metabolism, as established in studies on rodents, are summarized in Figure 3. Pyridine N-oxidation results in NNN-1-N-oxide (*1*). Hydroxylation of the 2′- and 5′-positions of NNN gives the unstable intermediates *3*–*6* which undergo non-enzymatic reaction with H_2O followed by

PM3001192373

Source: https://www.industrydocuments.ucsf.edu/docs/krwk0001

Table I. Levels of NNN and its metabolites in arterial and venous serum of miniature pig 3 at various intervals after injection of [5-^{3}H]NNN

	Time (min)	pmol/ml serum			
		NNN	Norcotinine (*2*)	Keto acid (*9*)	Hydroxy acid (*10*)
Arterial serum	0.5	1550	ND	18	ND[a]
	1.0	1540	ND	19	22
	2.0	997	18	26	96
	10.0	398	16	20	75
	15.0	343	17	27	75
	30.0	285	17	27	70
	60.0	235	31	44	92
	120.0	109	44	31	68
	180.0	97	47	32	53
Venous serum	0.5	920	ND	18	ND
	1.0	866	13	16	10
	2.0	784	17	29	65
	10.0	553	20	36	78
	15.0	467	21	37	85
	30.0	353	32	45	91
	60.0	246	40	49	110
	120.0	237	47	52	106
	180.0	163	36	31	94
	220.0	108	47	26	80

[a]ND = not detected.

Table II. Levels of NNN and its metabolites in tissues of miniature pig 3, 220 min after injection of [5-^{3}H]NNN

Tissue	pmol/mg dry weight			
	NNN	Norcotinine (*2*)	Keto acid (*9*)	Hydroxy acid (*10*)
Liver	0.11	0.28	0.13	0.54
Lung	0.50	0.27	0.11	0.26
Esophagus	0.55	0.23	0.05	0.30
Pancreas	0.46	0.12	ND[a]	0.38
Adrenal	0.90	0.11	0.08	0.26
Kidney	0.60	0.37	0.30	1.4
Mandibular glands	54.0	1.2	0.21	0.31
Harder's gland	67.2	4.3	0.20	0.36
Nose	1.9	0.7	ND	1.3
Snout	2.2	0.17	0.07	0.20

[a]ND = not detected.

Table III. Levels of NNN and its metabolites in urine and bile of miniature pig 3

	pmol/ml			
	NNN	Norcotinine (*2*)	Keto acid (*9*)	Hydroxy acid (*10*)
Urine	14.4	98.4	200	1110
Bile	64.7	ND[a]	500	731

[a]ND = not detected.

oxidation to give the keto acid *9* and hydroxy acid *10*. The latter two metabolites are considered to be good indicators of the initial extents of 2′-hydroxylation and 5′-hydroxylation of NNN respectively. Norcotinine (*2*) is presumed to arise by denitrosation and oxidation of NNN but this has not been established unambiguously; it could also be formed by an initial 5′-oxidation followed by denitrosation.

The disappearance of NNN from the arterial and venous serum of miniature pig 3 is illustrated in Figures 4A and 5A. An initial rapid clearance of NNN during the first 10 minutes was followed by slower clearance during the period from 10 to 220 min.

Table IV. Levels of NNN and its metabolites in stomach contents of miniature pig 3 at various intervals after injection of [5-^{3}H]NNN[a]

Time (min)	pmol/ml			
	NNN	Norcotinine (*2*)	Keto acid (*9*)	Hydroxy acid (*10*)
10	196	ND[b]	ND	ND
30	177	ND	ND	ND
45	599	18	5.2	NQ[c]
60	570	24	9.6	NQ
120	241	21	11	13
220	90	12	ND	18

[a]Stomach contents were withdrawn at intervals during the experiment.
[b]ND = not detected.
[c]NQ = not quantified due to interfering peak.

Similar results were obtained with pig 2 (data not shown).

Levels of NNN and its metabolites in the arterial and venous serum of pig 3 are summarized in Table I; the appearance of metabolites is illustrated in Figures 4B and 5B. The keto acid *9* could be detected in the arterial and venous serum, 0.5 min after injection of NNN. One minute after injection, the hydroxy acid *10* and norcotinine (*2*) were also detected. Relatively small changes in the levels of metabolites *2*, *9* and *10* occcurred during the period from 2 to 220 min. No other metabolites of NNN were consistently detected. The formation and persistence of NNN metabolites in the arterial and venous serum of pig 2 were similar to those in pig 3 (data not shown).

As shown in Table I, levels of hydroxy acid *10*, the end product of 5′-hydroxylation of NNN, exceeded those of keto acid *9*, the end product of 2′-hydroxylation at all time points from 2 to 220 min. The ratio of 2′-hydroxylation to 5′-hydroxylation as measured by the levels of these metabolites varied from 0.27 to 0.60 in arterial serum and from 0.33 to 0.49 in venous serum in the period from 2 to 220 min.

Table II summarizes levels of NNN and its metabolites in various tissues of pig 3, 220 min after injection. Concentrations of NNN in the mandibular gland and in Harder's gland greatly

PM3001192374

Source: https://www.industrydocuments.ucsf.edu/docs/krwk0001

exceeded those in all other tissues examined. These tissues also had relatively high levels of norcotinine. As in the serum, levels of hydroxy acid *10* exceeded those of keto acid *9* in all tissues examined. Levels of NNN and its metabolites in urine, bile and stomach contents of pig 3 are summarized in Tables III and IV.

Discussion

Previous studies of the metabolism of NNN *in vivo* have been carried out in CD-1 mice, F344 rats, Syrian golden hamsters and baboons (8,10–14). In those studies, the clearance of NNN from blood was investigated, and 24–48 h urinary metabolites were identified and quantified. The present experiments are the first in which the appearance of NNN metabolites in blood has been measured *in vivo*. The results clearly show that NNN is rapidly metabolized; the keto acid *9* was detected after only 30 s. The relatively constant levels of metabolites *2*, *9* and *10* during the 2–220 min period probably represents a steady-state between their formation and excretion into urine and bile.

Following a s.c. dose of 17 μmol/kg, the major 48 h urinary metabolites of NNN in the F344 rat were keto acid *9* (31% of the dose) and hydroxy acid *10* (37%). NNN-1-N-oxide (*1*, 7%), norcotinine (*2*, 3%) and NNN (3%) were also detected along with trace amounts of 3′-hydroxy-NNN and 4′-hydroxy-NNN (8,12,13). In the present study, we did not consistently detect any radioactivity coeluting with standard NNN-1-N-oxide. These results indicate that the miniature pig does not readily carry out pyridine-N-oxidation of NNN, in contrast to the F344 rat. In other respects, the overall metabolism of NNN by the F344 rat and miniature pig appear to be qualitatively similar since both species form metabolites *2*, *9* and *10* resulting from denitrosation and 2′- and 5′-hydroxylation.

Mutagenicity assays of stable precursors to the intermediates *5* and *6* indicate that *5* is highly mutagenic and suggest that metabolism of NNN by 2′-hydroxylation is a likely pathway of activation (15). Metabolism studies of NNN using cultured rat nasal mucosa and esophagus, which are target tissues for NNN carcinogenesis, have shown a predominance of 2′-hydroxylation over 5′-hydroxylation, which also supports the role of the former pathway in its metabolic activation (16,17). However, 5′-hydroxylation predominates in mouse lung, which is also a target tissue of NNN (18). The present study indicates that the miniature pig is capable of 2′-hydroxylation of NNN and thus suggests that it may be susceptible to NNN carcinogenesis. 5′-Hydroxylation exceeded 2′-hydroxylation at all intervals studied.

Whole-body autoradiography showed an accumulation of radioactivity in Harder's gland and the mandibular glands. H.p.l.c. analysis demonstrated that there were remarkably high levels of NNN in these tissues, indicating that the miniature pig concentrates NNN in these glands. The accumulation of NNN in the submaxillary and lacrimal glands of the mouse and rat has been observed previously and it has been suggested that this results from association of NNN with mucopolysaccharides (19,20). Other nitrosamines, such as *N*-nitrosopyrrolidine, *N*-nitrosodibutylamine, *N*-nitrosodiethylamine and *N*-nitrosobenzylmethylamine also are accumulated in lacrimal or salivary glands (21–24). Accumulation of NNN in the melanin of the eyes and skin has also been observed previously. The basis for these observations requires further study.

The presence of bound radioactivity in the nasal mucosa of the miniature pig is consistent with previous studies of NNN binding in rats and mice (19,20). The rat nasal mucosa has a high capacity for metabolism of NNN to intermediates which bind to DNA and protein and is a target tissue for NNN carcinogenicity (3,16,19). The present results thus suggest that the nasal mucosa of the miniature pig may be susceptible to the carcinogenic effects of NNN. Bound radioactivity was also detected in the tracheo-bronchial, esophageal, and oral mucosa of rats (19), which contrasts to the results obtained in the miniature pig.

Although NNN was administered by injection into the heart, it accumulated in the stomach contents during the first 60 min of the experiment. Thus, levels of NNN in stomach contents were greater than those in arterial or venous serum, 60 min after injection. Accumulation of nicotine, tobacco-specific nitrosamines, and their metabolites in stomach contents has been previously observed and is most likely due to their basicity (19,20,25,26). The stomach could act as a slow-release tissue for these compounds. In addition, it seems likely that nicotine and nornicotine would accumulate in the stomach of smokers, leading to endogenous formation of tobacco-specific nitrosamines under the favorable acidic conditions.

Acknowledgements

This study was supported by NCI Grant CA-44377. We thank Dr Staffan Eksbor for helpful discussions on the metabolism data. This paper is No. 110 in the series 'A Study of Chemical Carcinogenesis'.

References

1. Hofffmann,D. and Hecht,S.S. (1985) Nicotine-derived *N*-nitrosamines and tobacco related cancer: current status and future directions. *Cancer Res.*, **45**, 935–944.
2. Hecht,S.S., Castonguay,A., Rivenson,A., Mu,B. and Hoffmann,D. (1983) Tobacco specific nitrosamines: carcinogenicity, metabolism and possible role in human cancer. *J. Environ. Health Sci. CI* **1**, 1–54.
3. Brittebo,E.B. and Tjälve,H. (1983) Metabolism of *N*-nitrosamines by the nasal mucosa. In Reznik,G. and Stinson,S.F. (eds), *Nasal Tumors in Animals and Man*, Volume IV, *Experimental Nasal Carcinogenesis*. CRC Press, Boca Raton, FL, pp. 223–250.
4. Bustad,L.K. (1966) Pigs in the laboratory. *Sci. Am.*, **214**, 94–100.
5. Harrington,G.W., Magee,P.N., Pylypiw,H.M.,Jr, Bevill,R.V., Nelson,D.R. and Thurman,J.C. (1987) The pig as an animal model for the study of nitrosamine metabolism. In Batsch,H., O'Neill,I. *et al.* (eds), *Proceedings of the 9th International Meeting on N-Nitroso Compounds: Relevance to Human Cancer*. International Agency for Research on Cancer, Lyon, France, in press.
6. Hu,M., Bondinell,W.E. and Hoffmann,D. (1974) Synthesis of carbon-14 labelled myosmine, nornicotine and *N*′-nitrosonornicotine. *J. Labelled Compd.*, **10**, 79–88.
7. Ullberg,S. (1977) The technique of whole body autoradiography: cryosectioning of large specimens. Science Tools, The LKB Instrumental Journal, Special Issue on Whole Body Autoradiography, pp. 2–29.
8. Hecht,S.S., Lin,D. and Chen,C.B. (1981) Comprehensive analysis of urinary metabolites of *N*′-nitrosonornicotine. *Carcinogenesis*, **2**, 833–838.
9. Carmella,S.G. and Hecht,S.S. (1985) High-performance liquid chromatographic analysis of metabolites of the nicotine derived nitrosamines, *N*′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Anal. Biochem.*, **145**, 239–244.
10. Adams,J.D., LaVoie,E.J., O'Mara-Adams,K.J., Hoffmann,D., Carey,D.K. and Marshall,M.V. (1985) Pharmacokinetics of *N*′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in laboratory animals. *Cancer Lett.*, **28**, 195–201.
11. Adams,J.D., LaVoie,E.J. and Hoffmann,D. (1985) On the pharmacokinetics of tobacco-specific *N*-nitrosamines in Fisher rats. *Carcinogenesis*, **6**, 509–511.
12. Chen,C.B., Hecht,S.S. and Hoffmann,D. (1978) Metabolic α-hydroxylation of the tobacco specific carcinogen *N*′-nitrosonornicotine. *Cancer Res.*, **28**, 3639–3645.
13. Hecht,S.S., Chen,C.B. and Hoffmann,D. (1980) Metabolic β-hydroxylation and *N*-oxidation of *N*′-nitrosonornicotine. *J. Med. Chem.*, **23**, 1175–1178.
14. Hoffmann,D., Castonguay,A., Rivenson,A. and Hecht,S.S. (1981) Comparative carcinogenicity and metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N*′-nitrosonornicotine in Syrian golden hamsters. *Cancer Res.*, **41**, 2386–2393.
15. Hecht,S.S. and Lin,D. (1986) Comparative mutagenicity of 4-(carbethoxynitrosamino)-4-(3-pyridyl)-1-butanol and 4-(carbethoxynitrosamino)-1-(3-

PM3001192375

Source: https://www.industrydocuments.ucsf.edu/docs/krwk0001

pyridyl)-1-butanone, model compounds for α-hydroxylation of N'-nitrosonornicotine. *Carcinogenesis*, **7**, 611–614.
16. Brittebo,E.B., Castonguay,A., Furuya,K. and Hecht,S.S. (1983) Metabolism of tobacco specific nitrosamines by cultured rat nasal mucosa. *Cancer Res.*, **43**, 4343–4348.
17. Hecht,S.S., Reiss,B., Lin,D. and Williams,G.M. (1982) Metabolism of N'-nitrosonornicotine by cultured rat esophagus. *Carcinogenesis*, **3**, 453–456.
18. Castonguay,A., Lin,D., Stoner,G.D., Radok,P., Furuya,K., Hecht,S.S., Schut,H.A.J. and Klaunig,J.E. (1983) Comparative carcinogenicity in A/J mice and metabolism by cultured mouse peripheral lung of N'-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and their analogues. *Cancer Res.*, **43**, 1223–1229.
19. Brittebo,E.B. and Tjälve,H. (1981) Formation of tissue-bound N'-nitrosonornicotine metabolites by the target tissues of Sprague–Dawley and Fisher rats. *Carcinogenesis*, **2**, 959–963.
20. Brittebo,E. and Tjälve,H. (1980) Autoradiographic observations on the distribution and metabolism of N'-[^{14}C]nitrosonornicotine in mice. *J. Cancer Res. Clin. Oncol.*, **98**, 233–242.
21. Brittebo,E., Löfberg,B. and Tjälve,H. (1981) Extrahepatic sites of metabolism of N-nitrosopyrrolidine in mice and rats. *Xenobiotica*, **11**, 619–625.
22. Brittebo,E.B. and Tjälve,H. (1982) Tissue-specificity of N-nitrosodibutylamine metabolism in Sprague–Dawley rats. *Chem.-Biol. Interactions*, **38**, 231–242.
23. Brittebo,E.B., Löfberg,B. and Tjälve,H. (1981) Sites of metabolism of N-nitrosodiethylamine in mice. *Chem.-Biol. Interactions*, **34**, 209–221.
24. Kraft,P.L. and Tannenbaum,S.R. (1980) Distribution of N-nitrosomethylbenzylamine evaluated by whole-body radioautoradiography and densiometry. *Cancer Res.*, **40**, 1921–1927.
25. Hansson,E. and Schmiterlöw,C.G. (1962) Physiological disposition and fate of ^{14}C-labelled nicotine in mice and rats. *J. Pharmacol. Exp. Ther.*, **137**, 91–102.
26. Castonguay,A., Tjälve,H. and Hecht,S.S. (1983) Tissue distribution of the tobacco-specific carcinogen 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone, and its metabolites in F344 rats. *Cancer Res.*, **43**, 630–638.

Received on July 10, 1987; accepted on August 11, 1987

PM3001192376

Source: https://www.industrydocuments.ucsf.edu/docs/krwk0001